the surface of the crystal. Fine cracks, set along the spark tracks, quite distinct from the pitting, and bent in cleavage directions, add to the curious appearance in the field of the microscope. Sometimes a track approximating to those obtained on glass is met with, but the side rays are bent and distorted. I found, also, that the appearance of the tracks varied with the direction which the spark took in reference to the cleavage lines.

A similar experiment on a plate of selenite gave like results, the pits being more elongated in accordance with the more acute cleavage intersection. Mica plates showed a fine net of hexagonally arranged lines covering the surface where the spark had passed. Further experiments in this direction might be of interest, but other work has hindered me from pursuing the subject.

(25.) Sparks from the Leyden jar will produce tracks on glass similar to those described in the foregoing; but experimenting with such sparks is difficult, as their very explosive nature leads to a rapid break up of the cover glass.

January 9, 1890.

Sir G. GABRIEL STOKES, Bart., President, in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read:—

I. "New Experiments on the Question of the Fixation of Free Nitrogen. (Preliminary Notice.)" By Sir J. B. Lawes, Bart., LL.D., F.R.S., and Professor J. H. Gilbert, LL.D., F.R.S. Received (in part) and read January 9, 1890.

Received January 9.

In a paper presented to the Royal Society in 1887—1888, and printed in vol. 180 of the 'Philosophical Transactions,' we discussed the history and the present position of the question of the sources of the nitrogen of vegetation. We referred to the conclusions arrived at about thirty years ago from the results of Boussingault and from those obtained at Rothamsted, up to that time. We gave the results of some experiments which had been recently made at Rothamsted in connexion with the subject, and reviewed the evidence and conclusions of others published within the last few years.

It was considered that the earlier results obtained by Boussingault,

and at Rothamsted, under conditions in which the action both of electricity and of microbes was excluded, were conclusive against the supposition that, under such conditions, the higher chlorophyllous plants can directly fix the free nitrogen of the atmosphere, either by their leaves or otherwise. Others have, indeed, come to the conclusion that at any rate some plants do directly fix the free nitrogen of the air by their leaves. We believe, however, that there is, up to the present time, no evidence which can be held to be conclusive in favour of such a view.

It was pointed out how large was the store of already existing combined nitrogen in many soils and subsoils, and evidence was adduced to show that even *Leguminosæ* derive, at any rate a considerable amount of nitrogen from nitric acid within the soil and subsoil; and, further, that it was, as a rule, those having the most powerful root-development that took up the most nitrogen from somewhere; and it was considered that this fact pointed to a subsoil source.

Upon the whole it was concluded that, at any rate in the case of our gramineous, our cruciferous, our chenopodiaceous, and our solaneous crops, atmospheric nitrogen was not the source. It was admitted, however, that existing evidence was insufficient to explain the source of the whole of the nitrogen of the *Leguminosæ*.

According to some of the more recent experimenters, the fixation of free nitrogen is not limited to our leguminous crops; and the modes of explanation of the gains of nitrogen observed are extremely various. Thus, it is assumed—that combined nitrogen has been absorbed from the air, either by the soil or by the plant; that there is fixation of free nitrogen within the soil by the agency of porous and alkaline bodies; that there is fixation by the plant itself; that there is fixation within the soil by the agency of electricity; and, finally, that there is fixation under the influence of micro-organisms within the soil, with, or even without, the accompanying growth of higher plants.

The balance of the evidence recorded seemed to be undoubtedly in favour of the supposition that there is fixation under the influence of micro-organisms or of other low forms within the soil, and of all the various results which were discussed, those of Hellriegel and Wilfarth were considered to be by far the most definite and significant; pointing to the conclusion that, although the higher chlorophyllous plants may not directly utilise the free nitrogen of the air, some of them, at any rate, may acquire nitrogen brought into combination under the influence of lower organisms, the development of which is, apparently, in some cases, a coincident of the growth of the higher plant whose nutrition they are to serve. Such a conclusion is, however, of such fundamental importance that it seemed very desirable that it should be confirmed by independent investigation.

Accordingly, as stated in a postscript to our paper, dated October, 1888, it had been decided to institute experiments at Rothamsted on similar lines, and a preliminary series was then in progress. A second and more extended series has been conducted in the past season, 1889. It is proposed to give, on the present occasion, a description, and some of the numerical results, of the experiments made in 1888, and a description, and some illustrations, of the growth in those of 1889.

It was in 1883 that Hellriegel commenced a comprehensive series of vegetation experiments in pots, in which he grew agricultural plants of various families in washed quartz sand. To all of the pots nutritive solutions containing no nitrogen were added; to one series nothing else was supplied; to a second a fixed quantity of nitrogen as sodium nitrate; to a third twice as much; and to a fourth four times as much was added. The result was that, in the case of the Gramineæ, and some other plants, the growth was largely proportional to the nitrogen supplied, whilst in that of the Papilionaceæ it was not so. In the case of these plants, that of peas for example, it was observed, however, that in a series of pots to which no nitrogen was added, most of the plants were apparently limited in their growth by the amount of nitrogen which the seed supplied; whilst here and there a plant growing ostensibly under the same conditions would develop very luxuriantly; and, on examination, it was found that whilst no nodules were developed on the roots of the plants of limited growth, they were abundant on those of the plants that grew luxuriantly.

In view of this result, Hellriegel instituted experiments to determine whether, by the supply of the organisms, the formation of the root-nodules, and luxuriant growth, could be induced, and whether by their exclusion the result could be prevented. To this end, he added to some of a series of experimental pots 25 c.c., or sometimes 50 c.c., of the turbid extract of a fertile soil, made by shaking a given quantity of it with five times its weight of distilled water. In some cases, however, the extract was sterilised. In those in which it was not sterilised there was almost uniformly luxuriant growth, and abundant formation of root-nodules; but with sterilisation there was no such result. Consistent results were obtained with peas, vetches, and some other Papilionaceæ; but the application of the same soil-extract had no effect in the case of lupins, serradella, and some other plants of the family which are known to grow more favourably on sandy than on loamy or rich humus soils. Accordingly, he made a similar extract from a diluvial sandy soil where lupins were growing well, in which it might be supposed that the organisms peculiar to such a soil would be present; and, on the application of this to nitrogen-free soil, lupins grew in it luxuriantly, and nodules were abundantly developed on their roots.

The Experiments at Rothamsted in 1888.

This preliminary series comprised experiments with peas, blue lupins, and yellow lupins. The peas were grown—

1. In washed sand, with the ashes of the plant added; but no supply of combined nitrogen beyond a small determined amount in the washed sand and that in the seed sown.

2. In similarly prepared sand, but seeded with 25 c.c. of the turbid watery extract from a rich garden soil.

3. Duplicate of No. 2.

4. In the rich garden soil itself.

Each of the two descriptions of lupin was grown—

1. In sand prepared as for the peas, but with lupin-plant-ash instead of pea-plant-ash added.

2. In the same washed sand, &c., but seeded with 25 c.c. of the turbid watery extract from a sandy soil where lupins had grown luxuriantly.

3. In the lupin sandy soil itself.

4. In rich garden soil.

The twelve pots were arranged in a small greenhouse; and distilled water, free from ammonia, was used for watering.

The sand employed was a yellow sand from Flitwick, in Bedfordshire, and was of the same description as is used by gardeners in the neighbourhood for potting. It proved, however, not to be a very pure sand. Thus, after the stones and coarser portions had been removed by sifting, the remainder was several times washed, first with well-water and afterwards with distilled, the turbid washings being poured off; yet it was found to contain after being dried finally for a short time in a water-bath, and mixed with the plant-ash as mineral food, nitrogen as under :—

	Per cent. nitrogen.
Determined by soda lime	0·00287
Determined by copper oxide........	0·00245
Mean..............	0·00266

The sandy soil in which lupins had grown, and from which the watery extract was made for seeding the pots where the lupins were to grow, was still less pure; and it, of course, was not washed, and was only dried at about 24° C.; and, excepting that visible organic matter was removed by sifting and picking, it was used in its natural state as received. Duplicate determinations of nitrogen were made by soda-lime, in the lupin sand alone, and as used after mixture with the lupin ash. The following are the mean results, in each case, calculated on the dry sand :—

	Per cent. nitrogen.
In lupin sand, alone	0·0863
In lupin sand, with blue lupin ash.......	0·0826
In lupin sand, with yellow lupin ash	0·0888
Mean................	0·0859

It may be stated that, in this country, lupins are only grown as an agricultural crop, as food for sheep, on poor, sandy soils, on which little or nothing else will grow. The sand obtained for the purposes of the experiments was from land which had been reclaimed from a common in Suffolk, and on which no corn crop would grow; but on which, when subsequently sown with blue lupins, they had grown as high as the hurdles. It is stated, however, that lupins grow better on good land, but that they are grown on sandy wastes because they will thrive on them when no other crop will.

The garden soil, in the condition as analysed, contained 10·12 per cent. of moisture, and two determinations of nitrogen by soda-lime gave 0·3902 and 0·3936, mean 0·3919, corresponding to 0·4360 per cent. on the soil dried at 100° C.

The pots used were made of glazed earthenware; and were about 7 inches high, 6 inches in diameter at the top, and 5½ inches at the bottom, inside. They had a hole half an inch in diameter at the bottom for drainage, and another at the side near the bottom, into which, outside, a glass tube bent upwards was fixed for aëration; the tube being lightly closed with cotton-wool to prevent insects getting in. The pots rested on slips of thick sheet glass, placed in basins of the same glazed earthenware as the pots.

The mineral nutriment used was as follows:—For the peas, a mixture of 6 parts of pea-straw-ash and 1 part of pea-corn-ash; for the blue lupins, a mixture of 3 parts of blue lupin-straw-ash and 1 part of blue lupin-corn-ash; for the yellow lupins, a mixture of 4 parts of yellow lupin-straw-ash and 1 part of yellow lupin-corn-ash. In each case the greater part of the mixed ash was suspended in distilled water, and sulphuric acid added until there was a slight acid reaction; the rest of the ash was then added, and the whole evaporated to dryness and re-ignited. The ash was then very slightly alkaline to litmus. The so-prepared ashes were mixed at the rate of 0·5 per cent. with the greater part of the sand put into the pots; the remainder of the sand at the top of the pot being without ash.

The drain hole at the bottom of each pot was loosely covered with a piece of thick glass, 1 lb. of broken, washed, and dried flint was then put in, next the sand with ash, and lastly the sand without ash. The pots held from 7 to 9 lbs. of the yellow Flitwick sand, from 6 to 7 lbs. of the lupin sand, and about 4½ lbs. of the garden soil.

The soil extracts, supposed to supply the organisms, were made by shaking, in a large stoppered bottle, 1 part of the garden soil or lupin sand with 5 parts of distilled water; and after the heavier portions had settled down, syphoning off the turbid liquid, which was then passed through platinum gauze to separate any floating matter. The liquid was again shaken before taking the quantity required for seeding the soils, or for analysis. Determinations of nitric nitrogen by Schlœsing's method, and of total nitrogen by copper oxide, gave the following results:—

	Per cent.		In 25 c.c. extract.	
	Nitric nitrogen.	Total nitrogen.	Nitric nitrogen.	Total nitrogen.
	Per cent.	Per cent.	Milligram.	Milligram.
Garden soil extract....	0·000371	0·003159	0·093	0·790
Lupin sand extract....	0·000110	0·001184	0·028	0·296

It is thus seen that the 25 c.c. of the garden soil extract used for seeding contained little more than $\frac{3}{4}$ of a milligram, and the 25 c.c. of the lupin sand extract little more than $\frac{1}{4}$ of a milligram of nitrogen; quantities which are quite immaterial considered as a supply of combined nitrogen.

The Seeds.

The peas were of the description known as Maple field-peas. Four lots, each of 100 seeds, weighed 27·554, 27·460, 27·218, and 27·506 grams; giving an average weight per seed of 0·2743 gram. A large number of single seeds was then weighed, and those only retained for sowing or analysis which gave within 5 milligrams of the mean weight.

In the case of the blue lupins the largest and smallest seeds were picked out and rejected. Of the remainder, four separate lots of 100 each weighed 19·2290, 19·9215, 18·7960, and 19·4580 grams, giving an average weight per seed of 0·1935 gram. A large number of single seeds was then weighed, and those only kept for use the weight of which was within 5 milligrams of the average weight.

From the yellow lupin seed the largest were removed by sifting, and the smallest and those of a dark colour were picked out. Of the remainder, three separate lots of 100 each weighed 12·1060, 11·9640, and 11·6180 grams, giving an average weight per seed of 0·1190 gram. From these, seeds were selected for use which weighed within 5 milligrams of the average weight.

Determinations of dry matter, and of nitrogen, in the seeds, gave the following results:—

	Dry matter at 100° C.	Nitrogen.					
		In fresh.				In dry matter.	
		By soda-lime.			By copper oxide.	By soda-lime. Mean.	By copper oxide.
		Expt. 1.	Expt. 2.	Mean.			
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Maple peas..	93·26	3·537	3·621	3·579	3·531	3·837	3·787
Blue lupins..	94·03	5·105	5·098	5·101	5·364	5·425	5·705
Yellow lupins	94·63	6·649	6·569	6·609	6·404	6·984	6·767

It should be stated, as applicable to the whole of the results as well as to those recorded in the foregoing table, that nitrogen was determined by burning in a vacuum with copper oxide, and collecting and measuring the nitrogen and nitric oxide. In all cases, however, where there was sufficient material, determinations were also made by the soda-lime method, as a check. Nitrogen as nitrates was determined by Schlœsing's method. The copper oxide determinations given in the table, which are those used in the subsequent calculations, were made upon three or four of the average selected seeds, ground up with the copper oxide; whilst the check soda-lime determinations were made on quantities taken from a bulk of ground seeds.

The Vegetation Experiments in 1888.

It was intended to commence the experiments early in the summer, but the pressure of other work and the preparations necessary for the experiments themselves, prevented the sowing of the seed until early in August. Nevertheless, the results obtained in this initiative series were not only of value as affording experience on various points, of which advantage has been taken in the conduct of the more extended series made in 1889, but, as will be seen, they have afforded important evidence on the main point of enquiry itself.

The broken flints, the sand with ash, and the sand without ash, or the garden soil, as the case may be, were weighed and put into the respective pots at the laboratory, taken to the glass-house on August 4, and watered with ammonia-free distilled water. All the seeds were sown on August 6. Three accurately weighed seeds were put into each pot.

From the first, the peas germinated and grew well in each of the

four pots; but in each of the four pots of blue lupins, and in each of the four of yellow lupins, one or more seeds failed, and had to be replaced; and in some cases these also failed. There was in fact failure, not only in the poor Flitwick sand, but in the less poor lupin sand, and also in the rich garden soil. It is admittedly very difficult to secure healthy growth with lupins in pots. On discussing the matter with Hellriegel, at the meeting of the *Naturforscher Versammlung*, at Cologne, in September, 1888, he stated that it had required the experience of several years to insure favourable growth of lupins under such circumstances; and that one essential condition seemed to be that the soil must be kept open and porous; a result which, even with sand, was seldom attained if the dry materials were put into the pot, and then water poured on; the better plan being to bring the sand to a proper condition of moisture by well mixing water with it by degrees out of the pot, and then putting it lightly into the pot. It is also important that the mineral matter added to the soil should be quite neutral.

The failures are well illustrated by the photographs exhibited. Thus, in spite of the re-sowings, there were, on November 3, that is after three months since the first sowing of blue lupins, three plants in pot 1, with the yellow Flitwick sand without soil-extract; only two in pot 2, with the same sand and soil-extract seeding; none in pot 3 with the lupin sand itself, from which the soil-extract was prepared; and three, but of very varying size, in pot 4, with garden soil.

Then the photographs of the yellow lupins show that, in pot 1, with the yellow Flitwick sand, there remained only two plants; in pot 2, with the same sand and lupin soil-extract seeding, only two; in pot 3, with the lupin sand itself only two; and in pot 4, with the garden soil, only two plants.

We shall call attention to the development of the roots, and of nodules on them, in the case of the blue and yellow lupins, further on. Nitrogen determinations have also been made in most of the products; but, as with both blue and yellow lupins, there was actually less growth with than without the lupin sand extract, assumed to supply the organisms, we do not propose to discuss the analytical results on the present occasion; but, so far as that part of the subject is concerned, we shall confine attention to results relating to the peas, of which the growth was much more satisfactory, and the analytical results afford very important indications.

As already said, the peas in each of the four pots germinated and grew well. Throughout, those in the garden soil were more luxuriant than those in either of the other pots. Pots 2 and 3 were each seeded with 25 c.c. of the garden-soil-extract on August 13, that is just a week after the sowing of the seed. For some time, however, the plants in pot 1, with the sand without soil-extract, showed more

growth, and better colour, than those in either pot 2 or pot 3 with the soil-extract seeding. Indeed, it was not until about the middle of September, that is four or five weeks after the seeding with soil-extract, that the plants in pots 2 and 3 began to show a darker green colour than those in pot 1 without the soil-extract. The indication was, however, so striking, that on September 25 it was decided to count the leaves, and to estimate the relative area of leaf-surface, on the plants in the different pots. For this purpose, the leaves were classified into those which were dead, those that were dying, those which were changing colour, and those which were still bright green. It must suffice here to show the number, and the estimated relative area, of the total leaves in each case, on September 25, when the first counting and estimates were made, on October 17, on November 14, and on December 14, when the plants were cut. The following table summarises these results. The first four columns show the total number of leaves, and the second four the estimated relative leaf-surface, that of the plants of pot 1 (without soil seeding) on September 25, being taken as 100.

Peas, 1888.

	Number of leaves.				Estimated relative leaf-surface.			
	Pot 1.	Pot 2.	Pot 3.	Pot 4.	Pot 1.	Pot 2.	Pot 3	Pot 4.
September 25.......	144	140	120	164	100	67	58	128
October 17.........	188	200	184	216	143	172	158	242
November 14.......	244	300	244	280	170	249	245	328
December 14.......	382	540	390	434	267	481	434	463

It is thus seen that, on September 25, after it had been observed that the plants in pots 2 and 3, with the soil-extract seeding, had begun to show a darker green colour than those in pot 1 without the soil-extract, they nevertheless, up to that date, showed both a less number of leaves, and considerably less leaf-surface, than the plants in pot 1. It is not very clear why the plants with the soil-extract seeding should have remained so long in a comparatively backward condition. It may be that the result was only accidental, depending on the character of the seeds, or on the fact that pot 1 stood at the southern end of the row, and nearest the glass. The alternative is that, in the early stages of development of the organisms supplied in the soil-extract, and of the resulting nodules, the growth of the main plant was, in some way, retarded. The figures show, however, that, from

this date, the plants in pots 2 and 3 with the soil-extract, gradually gained upon those in pot 1 without it, both in number of leaves, and in leaf-surface; until, when the plants were taken up on December 14, those in pots 2 and 3 showed 540 and 390 leaves, against only 382 on those in pot 1; and the plants of pots 2 and 3 showed 481 and 434 of leaf-surface, against only 267 in pot 1. It is seen that there is here clear evidence of increased growth under the influence of the soil-extract seeding.

Photographs of the 4 pots of plants were taken on September 1, on September 22, on October 6, and lastly on November 3, about five weeks before the taking up of the plants, and they indicate relative progress consistently with the estimates given in the foregoing table.

In regard to the general character of growth it should be stated that, in all the pots, the upper portions of the plants obviously developed at the expense of the lower; the leaves of which gradually lost colour, and died off, whilst the stems and the leaves of the upper portion increased in growth; those in pots 2, 3, and 4, continuing to vegetate, and to maintain their bright green colour, up to the end; whilst those in pot 1 had shown more exhaustion, and maintained much less colour. There was, however, as was to be expected so late in the season, no indication of flowering in any of the pots.

It should be further stated, that the plants in all the pots commenced rather early to show signs of mildew, which increased very considerably, especially on the lower portions of the plants, in the later stages of growth. This was, perhaps, not to be wondered at, considering that the greenhouse was in the midst of allotment gardens, and that the plants were unavoidably subjected to considerable changes as to temperature and moisture of the atmosphere. Ventilation was, however, secured as far as practicable.

The next point to consider is, the actual and comparative development of the roots, and of nodules on them, in the different pots, with their different soil conditions. As the roots had to be preserved without any loss, for analysis, the mode of dealing with them for the purposes of examination had to be very carefully considered, and was necessarily more restricted than if examination had been the only object. After the above-ground growth had been cut off and removed, the pots, with their moist soil and roots, were kept in a warm dry place until the examination commenced. The block of soil was carefully turned out on to glazed cartridge paper, with as little shaking or disturbance as possible, and notes were at once taken as to the distribution of the roots, so far as it was then apparent. The sand or soil was then removed little by little, until the roots were left nearly bare. Further notes being then taken, the remaining sand or soil was removed as far as possible by washing in a beaker

with a little distilled water. The roots were then spread out upon paper, and so photographed, and finally noted upon.

Enlarged photographs of the roots of the plants grown in pot 1, with the yellow sand without soil-extract seeding, in pot 2, with the same sand and soil-extract seeding, and in pot 4, in the garden soil, were exhibited.

The roots in pot 1, with the yellow sand without soil-extract seeding, showed a densely matted mass of fibre, those of the different plants being considerably interwoven; and although a few fibres reached the bottom of the pot, and distributed through the flints, by far the greater portion was accumulated within the top 4 inches of the sand; and, notwithstanding there was here no soil-extract seeding, there were many nodules on the roots, but they were fewer, and generally much smaller, than on the roots grown with soil-extract seeding; they were also less characteristically accumulated near the surface, and more distributed along the root-fibres. There were, however, some agglomerations of nodules. Comparing this result with that obtained in 1889, with a purer and sterilised sand, there can be little doubt that the development of nodules, and the comparatively luxuriant growth, in this pot without soil-extract seeding, are to be attributed to the impurity, and non-sterilisation, of the sand.

The roots in pot 2, with soil-extract seeding, also showed a dense mass of fibre, which, however, extended from the top to the bottom of the soil, penetrated the layer of flints, and distributed over the bottom of the pot. In fact, the roots were much more generally distributed throughout the soil, and less accumulated within the surface layers, than in pot 1. The most developed root of the three, had three large agglomerated nodules, each with some scores of protuberances, somewhat as on a raspberry or mulberry. The other plants also showed similar nodules, but of a smaller size. There were also a number of small clusters distributed over the rootlets, but very few single nodules, differing in this respect from the character of development observed in pot 1.

In pot 3, also with soil-extract seeding, each of the three plants had developed a mass of root-fibre extending throughout the soil from the top to the bottom; though the greatest quantity was within the first 6 of the $7\frac{1}{2}$ inches of depth. There were large agglomerations of nodules on the roots of each plant. There were, besides, many small clusters, and here and there single nodules. By far the most of the nodules were within the top 3 inches of the sand; but one considerable bunch was found as low as 4 inches from the surface. As in the other cases, the nodules were grey, and much lighter in colour than the roots on which they grew.

Each of the three plants in pot 4, with the garden soil, had a

stouter main root than any of those in the other pots. From the side branches there proceeded a large amount of fine root-fibres, which extended throughout the whole soil, those from the different plants being much interwoven. The roots extended round the sides and along the bottom of the pot, much more than in either of the other pots. A photograph was, therefore, taken of the block of soil as it came out of the pot, showing this special character of root-development. There were three small clusters of nodules on the roots of each of the three plants, one or two smaller bunches, and here and there a single nodule. But the clusters were much smaller, the total number of nodules was much less, and they were more distributed throughout the soil, in this pot with rich garden soil, than in either of the others, even than in pot 1, without any soil-extract seeding. As the description shows, the root-development was at the same time much greater than in either of the other pots. To this point we shall have to recur again, but it may be remarked in passing, that the greater development of root and root-fibre, and the less development of the root-nodules, in the soil which itself supplied abundance of nitrogenous, as well as of other nutriment, is consistent with the observations of some other experimenters; but it is, on the other hand, inconsistent with the observations and views of others.

Finally in regard to the relative development of root-nodules under the different conditions, the evidence is clear, that there was a greatly enhanced development of them under the influence of the soil-extract seeding; and that, coincidently with this, there was a considerably increased growth of the above-ground parts of the plant.

The distinctly less development of root-nodules in the rich garden soil, than in the sand with soil-extract seeding, as observed in the case of the peas, was, however, not found in that of the lupins, as the following notes on the roots of the lupins grown in 1888, will show.

In pot 5, with the impure yellow sand, but without soil-extract, eventually three plants of blue lupins grew. From the short, thick, main root, many branches proceeded, extending from the top to the bottom of the soil; those plants having the largest above-ground development had also the most root. The branches were fleshy and succulent, and thicker at a distance from the main stem than near it. No nodules were observed on the roots in this pot.

In pot 6, with lupin-soil-extract, but with only two plants, the roots were of the same general character as to branching, extension, fleshiness, and succulence, as those in pot 5. There was, however, one nodule, about the size of a pea, on a root-fibre on one of the two plants.

In pot 7, with the lupin sand itself, there was no plant.

In pot 8, with the garden soil, there were three plants, two of them small ones from more recent sowings than the other, and with much less root development; but there were three or four nodules or swellings on the root-fibres of each plant. The largest and oldest plant showed a very great development of root, extending throughout the soil, round the sides, and along the bottom of the pot. On the main root, which was thick and strong down to about 5 inches, there were two large swellings or nodules about 3 inches from the surface, each of which, unlike the bunches of nodules on the pea roots, appeared externally to be single and solid, but indented. There were, besides, nineteen small swellings on the root-fibres, of the same colour as the root itself, and whether these were nodules or not was not very obvious.

In pot 9, in the yellow sand without soil-extract, there were two plants of yellow lupins. With less above-ground growth, there was also considerably less root development, than under the same soil conditions with the blue lupins (pot 5). As in the case of the blue lupins, there were, however, no nodules.

In pot 10, with lupin-soil-extract seeding, there were two small plants, also with small root-development, but throwing out much fine fibre near the surface, and then slender branches to the bottom of the pot. There were here, again, no nodules.

In pot 11, with the lupin sand, there were two plants, one very much larger than the other. There was a swelling on the thick main root of the smaller plant, but there were no nodules on the rootlets. The larger and older plant developed a dense mass of both fleshy and fine fibrous root. The main root, about 1 inch from the surface, was encased by a large swelling. The roots extended from the top to the bottom of the soil. No nodules were observed on the rootlets, but there was an abundance of root-hairs.

In pot 12, with garden soil, there were two plants. The stout, woody, main root, extended deeper than in the other pots; and there were many branches, extending round the sides and along the bottom of the pot. The larger plant had two swellings on the main root, about 1½ inch from the surface, each of the size of a field bean; also three small nodules on the root-fibres. The smaller plant had one indication of such a swelling on the main root, and twelve nodules on the root-fibres, three the size of a pea, three half as large, and six very small. The larger were 1 or 2 inches from the surface, the others lower, one 6 inches down. There was more fine fibre, but very much less development of root-hairs than in pot 11.

Thus, in the case of both blue and yellow lupins, there were no nodules without soil-extract, and only one with the lupin soil-extract seeding. In the lupin soil itself there was some indication, but in the garden soil there was, with both descriptions of lupin, a much

more marked development, both of swellings on the main roots, and of nodules on the root-fibres. The very meagre development of nodules both with lupin-soil-extract seeding, and in the lupin-soil itself, in 1888, when, as will be seen further on, the result was so different in 1889, suggests the question whether the lupin-sand of 1888 had been too much dried, and so sterilised.

The Analytical Results.

We will now turn to the evidence afforded by analysis, as to the difference in the amount of growth, and especially as to the difference in the amount of nitrogen assimilated, in the peas grown under the different conditions.

The following table shows the percentages of ash, and of nitrogen determined by copper oxide (each calculated on the dry substance), in the stems and leaves together, and in the roots, of the plants in the different pots.

		Per cent. in dry substance.			
		Ash.		Nitrogen.	
		In stems and leaves.	In roots.	In stems and leaves.	In roots.
		Per cent.	Per cent.	Per cent.	Per cent.
Pot 1.	Sand, without soil-extract	19·70	28·67	2·904	2·574
Pot 2.	Sand, with soil-extract..	16·07	36·75	4·900	3·195
Pot 3.	Sand, with soil-extract..	13·87	23·26	4·006	3·357
Pot 4.	Garden soil	9·17	20·44	4·534	2·791

It is remarkable how much lower is the percentage of ash in the dry substance of the more normally-developed plants grown in the garden soil, than in that of those grown in the sand with plant-ash added. There can be no doubt that the amount of soluble mineral matter provided in the quantity of ash used was excessive; and less has been supplied in the experiments of 1889. The percentage of ash in the dry substance of the roots is, however, in all cases high, but doubtless some adherent sand would be included.

The differences in the percentage of nitrogen in the dry substance of the differently grown plants are consistent with the known characters of growth. Thus, the lighter colour, and the comparatively restricted growth, of the plants in pot 1, indicated nitrogen exhaustion; and the

percentage of nitrogen in the dry substance, of both the above-ground and the under-ground produce, is lower than in either of the other cases. It may be further noted, that the roots grown in pots 2 and 3 with the soil-extract, and with so much greater development of nodules than in either pots 1 or 4, at the same time contained a considerably higher percentage of nitrogen in their dry substance.

The next table shows the actual quantities of dry substance, of ash, and of nitrogen, in the separated, and in the total, products of growth.

	Actual amounts in the produce.								
	Dry substance.			Ash.			Nitrogen.		
	In stems and leaves.	In roots.	In whole plant.	In stems and leaves.	In roots.	In whole plant.	In stems and leaves.	In roots.	In whole plant.
	grams.	grams.	grams.	grams.	gram.	grams.	gram.	gram.	gram.
Pot 1	7·423	2·600	10·023	1·462	0·745	2·207	0·2153	0·0669	0·2822
Pot 2	9·368	2·409	11·777	1·505	0·885	2·390	0·4591	0·0770	0·5361
Pot 3	9·411	1·748	11·159	1·305	0·407	1·712	0·3771	0·0587	0·4357
Pot 4	12·808	2·846	15·654	1·175	0·582	1·757	0·5816	0·0794	0·6600

It is seen that there is more dry substance in the above-ground growth, but less remaining in the roots, in pots 2 and 3 with the soil-extract than in pot 1 without it. In the whole plant there is, of dry substance with soil-extract, about $11\frac{3}{4}$ grams in pot 2, and more than 11 grams in pot 3, against only 10 grams in pot 1 without soil-extract.

The point of chief interest is, however, that there was twice, or more than twice, as much nitrogen in the above-ground growth in pots 2 and 3 with the soil-extract seeding, as in pot 1 without it. But there is much less difference in the amount of nitrogen remaining in the roots under the different conditions. In the total vegetable matter there is in pot 2 more than twice, and in pot 3 nearly twice, as much nitrogen as in pot 1 without the soil-extract.

With the full supply of already combined nitrogen in pot 4, with garden soil, there was about one and a third time as much dry substance produced, and more nitrogen assimilated, than under the influence of the soil-extract seeding.

The significance of the results relating to the nitrogen is, however, more clearly brought to view in the next table, which shows—the amounts in the soils at the commencement and at the conclusion of the experiment, and the gain or loss; the amounts in the seed, in the total products of growth, and the gain; the total nitrogen in the

soil and seed at the commencement, in the soil and produce at the conclusion, and the gain. Lastly, in the last column but one, the total gain, reckoning in each case, the initial nitrogen = 1; and, in the last column, the gain in the plants, reckoning the nitrogen in the seed = 1.

[The results relating to the soils in pots 1, 2, and 3, are calculated from the copper oxide determinations; which gave, in the dry sand at the commencement (as already shown) 0·00245 per cent. nitrogen; and at the conclusion the amounts were:—0·00269 in that of pot 1, 0·00239 in that of pot 2, and 0·00208 in that of pot 3. The determinations in the garden soil were by the soda-lime process, and were only made to obtain a general idea of the result, and were not intended for exact quantitative estimates of gain or loss. Thus, the higher the percentage of nitrogen, the smaller the quantity that can be taken for burning, whilst such a soil, rich from the application of dung, is a very heterogeneous mixture, and difficult to sample for analysis. It, moreover, contains a very large amount of carbon, and gives a coloured acid for titration. Nor was the nitric acid determined, either at the commencement or at the conclusion; but, with so much organic matter the error, if any, thus arising would be immaterial. The determinations were, however, fairly accordant for such material; giving, calculated on the dry soil, at the commencement, 0·4341 and 0·4379, mean 0·4360 per cent.; and at the conclusion, 0·4378 and 0·4342, mean 0·4360. It may be added, that a difference or error of 0·01 in the percentage of nitrogen in the soil, would represent a gain or loss of 0·204 gram on the quantity of the garden soil used. The data upon which the amounts of nitrogen in the seed, and in the products of growth, are calculated, have been already considered.—January 24, 1890.]

	Nitrogen.										
	In soil.			In seeds and produce.			Total.			In total products, total initial =1.	In plants, nitrogen in seed = 1.
	At commencement.	At conclusion.	Gain (+) or loss (−).	In seeds sown.	In total plants.	Gain.	At commencement.	At conclusion.	Gain.		
	grams.	grams.	gram.	gram.	gram.	gram.	grams.	grams.	gram.		
Pot 1...	0·0999	0·1096	+0·0097	0·0293	0·2822	0·2529	0·1292	0·3918	0·2626	3·03	9·63
Pot 2...	0·0999	0·0974	−0·0025	0·0298	0·5361	0·5063	0·1297	0·6335	0·5038	4·88	17·99
Pot 3 ..	0·0999	0·0848	−0·0151	0·0291	0·4357	0·4066	0·1290	0·5205	0·3915	4·04	14·97
Pot 4...	7·9989	7·9989	—	0·0301	0·6600	0·6299	8·0290	8·6589	0·6299	1·08	21·93

The first point to notice is, that there is very little difference in the amount of nitrogen in the soils at the commencement, and at the conclusion, of the experiments. There would, doubtless, be some

fine root-fibre not removed at the conclusion, so that where there is loss it is to be supposed that some of the original nitrogen of the soil has contributed to the growth. In the case of the garden soil, with its high percentage of nitrogen, it is of course not impossible that there may have been some loss by evolution of free nitrogen.

That there is at any rate no material gain in the soils would seem to be confirmatory of the conclusion indicated by other evidence, that the fixation of nitrogen is not effected by the organisms within the soil independently of the symbiotic growth of the nodules and their contents and the higher plant to which they are attached, to whose nitrogenous supply they seem to contribute. Indeed, if the fixation had taken place under the influence of microbes within the soil, independently of connexion with the higher plant, we should have to conclude that the latter had, nevertheless, availed itself of exactly the whole of the nitrogen so brought into combination—a supposition for which there would seem no reasonable justification.

Turning to the middle division of the table, which shows the nitrogen in the seed sown, in the total vegetable matter grown, and the gain, and disregarding the changes in the soil itself, which it has been seen may well be done, it will be observed that the gain of nitrogen in the plants is so large as to be very far beyond the limit of any possible experimental error. This certainly cannot be said of some of the experiments conducted on other lines, the results of which have been published in recent years, and been held to show the fixation of free nitrogen under the agency of micro-organisms within the soil, without coincident higher plant-growth, or with the coincident growth of other plants than of the leguminous family.

The gain in these initiative experiments with peas is, however, much less than in many of those of Hellriegel and Wilfarth. This is not to be wondered at, when the late period of the season, and the consequent character of the growth, are borne in mind; and when we come to consider the greater growth attained in the experiments of 1889, little doubt can be entertained that the fixation was then very much greater than it was in 1888.

To refer to the figures, it is seen that, whilst the nitrogen supplied in the seed was only 0·03 gram or less, that of the products of growth was 0·2822 gram in pot 1, 0·5361 in pot 2, 0·4357 in pot 3, and 0·6600 in pot 4; and the gains are $\frac{1}{4}$ of a gram in pot 1, more than $\frac{1}{2}$ a gram in pot 2, nearly $\frac{1}{2}$ a gram in pot 3, and more than $\frac{1}{2}$ a gram in pot 4.

The third division of the table shows—the total nitrogen at the commencement (in soil and seed together), at the conclusion (in soil and total vegetable matter grown), and the gains. But the significance of the results is more clearly seen in the last two columns. The first of these shows the relation of the amount of nitrogen in the total products (soil and plants together) to the total initial nitrogen (soil

and seed together), taken as 1. It is seen that, even in pot 1, with the impure and not sterilised sand, but without soil-extract, there was, so reckoned, more than three times as much nitrogen in the products as in the soil and seed; in pot 2, with soil-extract, there was about five times as much; and in pot 3, also with soil-extract, there was more than four times as much. In the case of pot 4, however, with garden soil, owing to the large amount of initial nitrogen in the soil, the gain, so reckoned, appears but small.

It is in the last column of the table, in which, disregarding the nitrogen in the soils, which remained so nearly constant throughout, and reckoning the relation of the nitrogen in the total products of growth to that in the seed taken as 1, that the large amount of fixation is brought clearly to view. So reckoned, the nitrogen in the substance grown was—in pot 1, 9½ fold; in pot 2, nearly 18 fold; in pot 3, nearly 15 fold; and in pot 4, nearly 22 fold, that supplied in the seed.

The Vegetation Experiments in 1889.

In this second season a more extensive series was arranged. The plants selected were—peas, red clover, vetches, blue lupins, yellow lupins, and lucerne. For the lupins and lucerne, specially made pots of glazed earthenware, about 6 inches in diameter, and 15 inches deep inside, that is about twice as deep as the pots used in 1888, and as used again for the peas, red clover, and vetches, were employed. These pots had holes at the bottom for drainage, and slits at the side, near the bottom, for aëration. All the pots stood in specially made saucers or pans of the same material. A quantity of broken, washed, and this time ignited flint, was put into the bottom of each pot. The sand used was a rather coarse white quartz sand, from which the coarser and the finer portions were removed by sifting, and more of the finer by washing and decantation, first with well, and afterwards with distilled water. In defect of means for igniting so large a quantity of material (about 300 lbs.) without running the risk of gaining more impurity than was expelled, the portion retained for use was kept, in successive lots, in a large water-bath, at nearly 100° C., for several days, and then preserved in well-closed bottles. The results will show that the sand so prepared was sufficiently, if not absolutely, sterilised.

In each case the sand was mixed with 0·1 per cent. of the plant-ash, and 0·1 per cent. of calcium carbonate.

There were four pots of each description of plant. Of the peas, clover, vetches, and lucerne, No. 1 was with the prepared quartz sand without soil-extract; No. 2 with the quartz sand and garden soil extract added; No. 3 was duplicate of No. 2; and No. 4 was with the garden soil itself. Of the blue and yellow lupins, No. 1 was with

the prepared quartz sand without soil-extract; No. 2 with lupin-soil-extract added; No. 3 was duplicate of No. 2; and No. 4 was with the lupin soil itself, to which 0·01 per cent. of the plant-ash was added.

The soil-extracts were in all cases added on July 9, before the sowing of the seed; 25 c.c. in the case of the peas, vetches, and clover, and 50 c.c. in that of the lupins and lucerne.

The seeds, carefully selected and weighed as in 1888, were sown on July 10, that is, about four weeks earlier than in the previous year, but still not so early as was desirable. In the case of the clover, ten seeds were sown in each pot; in that of the blue and yellow lupins three, and in that of the peas, vetches, and lucerne, only two seeds were put in each pot.

In all four pots, the peas germinated and grew well from the beginning. In the No. 1 pot of vetches, one seed failed and had to be replaced. Several of the red clover seeds failed, and eventually four plants only were left in each pot. As in 1888, most of the blue lupins failed; and eventually only one plant in only one of the four pots, remained. Some of both the yellow lupins and the lucerne also failed; but, as will be seen further on, eventually two good plants of each remained in each pot.

No analytical details relating to the experiments of 1889 are yet available; but the notes on growth, and the photographs of the plants and of their roots convey a clear idea of the importance and significance of the results obtained.

The peas were taken up on October 23 and 24. Photographs of the four pots of plants were taken on August 3, August 20, September 27, and October 22, that is the day before taking up; and an enlargement of the last taken is exhibited. It is seen that, unlike the result obtained in pot 1 in 1888 with the impure and non-sterilised sand, the plants in the purer and sterilised quartz sand, show extremely limited growth. Before the end of July, the plants in both pots 2 and 3, with soil-extract, began to show enhanced growth compared with that in pot 1, without soil-extract seeding; and eventually, whilst the plants in pot 1 were only $8\frac{1}{4}$ and $8\frac{1}{2}$ inches in height, those in pot 2 with soil-extract were 14 and $50\frac{1}{2}$ inches; and those in pot 3, also with soil-extract, were $52\frac{1}{2}$ and $50\frac{1}{2}$ inches high. In pot 4, with the garden soil, the plants showed even somewhat less extended growth than those in pots 2 and 3 with the soil-extract only. But the plants in pot 4 were more vigorous, and whilst they flowered and seeded, neither of those in either pot 2 or 3 did so; but continued to vegetate, the upper parts apparently at the expense of the lower.

The root development should be briefly noticed. In pot 1, without soil-extract, it was altogether much less than in either pot 2 or pot 3 with soil-extract, or than in pot 4 with garden soil. Enlarged photo-

graphs of the roots of pots 1, 3, and 4, clearly illustrate this. It is further seen that, in pot 1, without soil-extract, the main roots descended some distance before they threw out any considerable amount of root-branches and of root-fibre; whereas, in pots 2 and 3, with soil-extract, there was characteristically much more fibre distributed both in the upper layers and throughout the pot.

It is specially to be noted that, whereas in pot 1 in 1888, with impure and non-sterilised sand, there was a considerable development of nodules, now in the pure and sterilised sand, not a nodule was observable.

In pot 2, with soil-extract, one plant was very much larger than the other, and developed very much more root. The smaller plant had, however, several nodules on the main root, near the surface of the soil, and a good many small ones distributed along the fibres. Most of the nodules were more or less shrivelled. The larger plant had a large cluster of nodules on the main root, very near the surface; and a very large number of single nodules, mostly small, was distributed on the root-fibres, quite to the bottom of the pot. Upon the whole those on the larger plant were less shrivelled.

In pot 3, also with soil-extract, the main roots extended to, and along, the bottom of the pot; throwing off many side branches, with a very large quantity of fine fibrous root. The greatest distribution was, however, in the upper few inches of the soil. There were two clusters of nodules on one of the plants, and three on the other, besides smaller bunches. A large number of mostly single small nodules was also distributed along the roots. On one of the plants, the largest cluster was on the main root, and on the other the clusters were on the side branches.

In pot 4, with the garden soil, there was a dense mass of root-fibre throughout the first 6 inches of depth. There were numerous nodules, the majority single, and within the upper 2 or 3 inches of soil. There were also a few small bunches.

Thus, then, the limited growth in pot 1, without soil-extract, is coincident with the entire absence of nodule-formation; and the increased growth in pots 2 and 3, with soil-extract, is coincident with a very great development of nodules. In pot 4, with garden soil, itself supplying abundance of nitrogen, there was also a considerable development of nodules, but distinctly less than in pots 2 and 3, with soil extract only.

The vetches were taken up on October 26. They had been photographed on August 3, August 20, September 27, and lastly on October 25, that is, the day before taking up; and of this last representation an enlargement was exhibited.

Here, as with the peas, the plants in pots 2 and 3, with soil-extract, had shown more growth than those in pot 1 without it, before

the end of July. Again, as with the peas, the vetches in the pure and sterilised sand showed extremely limited growth. On the other hand, those in pots 2 and 3, with the soil-extract grew, as shown in the photograph, to a very great height; indeed, higher than those in pot 4 with the garden soil.

The heights of the plants were—in pot 1, without soil-extract, $11\frac{1}{4}$ and $10\frac{1}{2}$ inches; in pot 2, with soil-extract, $52\frac{1}{2}$ and 67 inches; in pot 3, also with soil-extract, $61\frac{1}{2}$ and 51 inches; and in pot 4, with garden soil, only 53 and 36 inches.

But, as in the case of the peas, whilst the plants in pot 4 with the garden soil flowered and seeded, those in pots 2 and 3, with the soil-extract only, did not, but continued to extend upwards at the expense of the lower parts of the plant.

There was much less development of root in pot 1, without soil-extract, than in either pots 2 or 3 with it, or than in pot 4 with the garden soil. The main roots descended to the bottom of the pot, and threw out a number of side branches, but there was a marked deficiency of root-fibre. Not a single nodule was found.

In pot 2, with soil-extract, there was, as shown in a photograph, a dense mass of root and root-fibre, which distributed throughout the whole of the soil, though the greatest accumulation was within the first 3 inches of depth. There were numerous nodules, but considerably less in quantity than on the corresponding pea-plants. They were mostly single, the greater number being found in the lower layers, which is also contrary to the result with the peas. They were, moreover, generally exceedingly small.

In pot 3, also with soil-extract, there was also an immense development of root and root-fibre through the whole area of the soil; the greatest accumulation being in the upper and lower portions of the pot, with less in the middle. There were many nodules, but very small, and probably fewer than on the roots in pot 2. All the nodules were single, and fairly distributed over the whole root area.

In pot 4, with garden soil, there was a moderate amount of root and of root-fibre, chiefly within the upper 6 inches of depth; but there was altogether very much less of root development than in either pots 2 or 3 with the soil-extract. There were many nodules, but all single, and very small; and they appeared to be flattened, as if exhausted of their contents.

Here again, then, as with the peas, the very restricted growth in pot 1, without soil-extract seeding, was associated with very limited root development, and with the entire absence of nodule-formation. On the other hand, the very greatly extended vegetative growth in pots 2 and 3, with soil-extract, was associated with an immense development of root and root-fibre, extending throughout the pots, and with the formation of numerous nodules; which, however, were generally

smaller, more distributed over the whole root area, and less accumulated near the surface, than in the case of the peas. Lastly, in the garden soil, with its liberal supply of combined nitrogen, there was much less development of roots, and less also of nodules, than in the pots with soil-extract only.

Received January 21.

It has already been said that most of the blue lupins failed; but it was with the yellow lupins that the most striking results were obtained.

As in the case of the other plants, the yellow lupin seeds were put in on July 10, three being sown in each pot. There were some re-sowings, some seeds taken out, and, eventually, two plants were left in each pot. By the end of July those in pots 2 and 3, with the lupin-soil-extract seeding, already showed more growth than those in pot 1 without it. Photographs were taken on August 3, August 20, September 27, October 28, and November 29; and the plants were cut on December 7. An enlargement of the photograph taken on October 28 was exhibited; and the later representation, that of November 29, was thrown on the screen.

It is seen that the plants in pot 1, without soil-extract seeding, scarcely appeared over the rim of the pot, one being only about $1\frac{1}{2}$, and the other about $2\frac{1}{2}$ inches high. In pot 2, with lupin-soil-extract seeding, one plant was about 2 feet, and the other more than $1\frac{1}{2}$ foot high; both spreading much beyond the width of the pot. In pot 3, also with lupin-soil-extract seeding, one plant was more than 2 feet, but the other little more than 8 inches high. In fact, in both these pots with soil-extract seeding only, the plants showed considerably more development than those in pot 4 in the lupin-soil itself; one of these being only about 16, and the other about 18 inches high, and both less branching than those in pots 2 and 3.

Unlike the peas and vetches, the yellow lupins with soil-extract seeding flowered and podded freely. One plant in pot 2 had nine small pods; and one in pot 3, four large and three small ones. There were also in pot 4, with lupin-soil, on one plant five pods, and on the other six.

Thus, in the quartz-sand with lupin-soil-extract seeding, the plants not only produced a great deal more vegetable matter than those in the lupin-sand itself, but they as freely flowered and seeded.

Photographs of the roots of the plants in each of the four pots were taken; and enlargements of those in pot 1 without soil-extract seeding, in pot 3 with soil-extract seeding, and in pot 4 with the lupin-soil itself, were exhibited.

In pot 1, without soil-extract, and very restricted above-ground growth, there was coincidently very little root development. The main roots descended far down the deep pot almost without branching, but at the bottom a number of branches, and a mass of fibre were produced. The root-fibres were fleshy and succulent. No root swellings or nodules were found.

In pot 2, with the lupin-soil-extract seeding, there was, on the other hand, a very great development of root. Branches were thrown out throughout the whole length; and at their ends masses of fleshy fibrils were formed, which were thickly coated with root-hairs. On the main root of one plant, 3 inches down, there was a large swelling or nodule the size of a field bean; 4 inches lower there were three smaller ones on a side branch; 10 inches down there were three as large as peas; and lower still there was another small swelling, more like the nodules found on other plants. The other plant had less root growth. One and a half inch down there was a swelling the size of a small pea; and $4\frac{1}{2}$ inches lower there were three swellings, one as large as a bean, and the others about the size of a vetch seed. These swellings on the lupin-roots, which were all on the main roots or thicker branches, are very different in appearance from the nodules on the pea and vetch-roots. They are, as described, swellings, encasing the root where they grow.

In pot 3, also with the soil-extract seeding, one plant, as an enlarged photograph shows, developed an immense amount of branching root, with a great deal of root-fibre, which extended throughout the whole soil, but to a greater degree in the lower than in the upper half of the pot. The main root was woody near the top. The lower root-fibres were fleshy, and thickly coated with root-hairs. There were several swellings or nodules on the main root below 5 inches; and lower down, on a root-branch, there were several swellings; there being in all twelve on this plant. On the smaller and more meagrely rooted plant, about 10 inches down, there were also two bunches of small nodules, and three single nodules; and a little lower, on a side branch, another small nodule. With regard to the great development of root-hairs on the fine fibrils of the roots in both pots 2 and 3, with quartz sand and soil-extract seeding, it may be supposed that this was an effort to acquire mineral nutriment, in quantity commensurate with the large amount of nitrogen fixed, and available to the plant.

In pot 4, with the lupin-sand, the distribution of root was very different from that in pots 2 and 3, with the soil-extract. The main root, at a depth of 2 inches, threw out many thread-like branches, at the end of each of which there was a bundle of fine fibre. The lower fibres became thicker, and were white and fleshy; but they were without the marked development of root-hairs observed in such

abundance in pots 2 and 3. Most of the root was within 6 inches of the surface, and there seemed to be none below 14 inches. One to two inches from the surface, there were swellings on the main roots which were less raised, but more spreading, than those on the roots in pots 2 and 3. There were also, on one side branch, six very small nodules.

To sum up in regard to the yellow lupins: Under the influence of the soil-extract seeding, the above-ground growth was not only very luxuriant, but the plants developed great maturing tendency, flowering and seeding freely. The development of the roots generally, and that of swellings or nodules on them, were also very marked; and there can be no doubt that the gain of nitrogen will be found to be very large. In pot 4, with the lupin-sand itself, which would supply a not immaterial amount of combined nitrogen, although the growth was normal, it was, both above ground and within the soil, very much less than in the pots with soil-extract only; and the development of nodules was also less. It is possible that the less development in the lupin-sand itself, than in the quartz-sand with soil-extract only, was partly due to the much less porosity of the lupin-soil, especially when watered. At any rate, the results with the soil-extract only are very remarkable.

As the main growth of red clover is in the second year, and that of lucerne also in years subsequent to the first, the pots of these plants are left for further growth; so that there is, at present, but little of definite result available in regard to them. There are, however, some points of special interest to notice.

A photograph of the clover plants taken on September 28 was thrown on the screen. The above-ground growth in pot 1, without soil-extract, was distinctly more than in either pots 2 or 3 with it; and it is judged that the amount of growth will probably prove to be greater than is to be accounted for by the amount of nitrogen supplied in the seed sown. As the soil-extract seeding in pots 2 and 3 seemed to be without effect, a second amount of extract, but this time from garden soil where clover was growing well, was, on September 4, applied to pot 2; but to pot 3 there was added instead a solution of calcium nitrate, and this application was continued up to December 6, when, in all, 0·23 gram of nitrogen had been so applied. The effect of the nitrate was, undoubtedly, some increased growth, but especially an increased depth of green colour. It remains to be seen what will be the final result.

The application of garden-soil-extract to lucerne also appeared to be entirely without effect up to the beginning of September; the plants in pot 1 without soil-extract, and those in pots 2 and 3 with it, showing no difference, and apparently no progress. On September 4, therefore, pot 2 was re-seeded with soil-extract, this time from a soil

growing lucerne; and, at the same time, a solution of calcium nitrate was added to pot 3, and the application was continued, as in the case of the clover. For many weeks the repeated soil-extract seeding was without any apparent effect; but, quite recently, there has been a slightly increased depth of colour, and perhaps a little growth. The application of nitrate to pot 3, however, showed marked effect very soon after the application had been commenced; and, as the representation of the growth on December 23 shows, there was up to that time, considerable growth under the influence of the nitrate.

The darkening of the colour of the leaves of the clover, and the increased growth of the lucerne, under the influence of the nitrate, in soil otherwise nitrogen-free, is of interest. Not that there is any want of abundant evidence showing that Leguminosæ do take up nitrogen largely as nitrate, but, in view of the new results under the influence of micro-organism seeding, it seems to be assumed by some that these plants probably depend for their nitrogen exclusively on such agency.

Before concluding in regard to the experimental plants, some reference should be made to the very great difference in the external appearance and character of the swellings, or nodules, on the roots of the different descriptions of plant, and even on those of the same description. In the course of the examinations this was so marked, that it was contemplated to take photographs illustrating the most characteristic differences; and, as it was found that the roots of the experimental plants, which had to be preserved for analysis, could not without risk be manipulated as required for the purpose, some plants were procured from the garden and the fields, and notes of previous observations were looked up. Presumably owing to the late period of the season, the roots so obtained were, however, not suitable for the illustration desired. It must suffice, therefore, avoiding any attempt at technical description, to make a few general observations on the facts at command; and to say that we hope to follow the subject up at a more suitable season of the year, and then to be able to give some account, not only of the general external, but, if possible, of the internal characters of the different bodies.

It should be stated that, so far as the nodules on the roots of the bean are concerned, a full technical description, both of their external characters and internal structure, has been given by Professor Marshall Ward ('Phil. Trans.,' B, 1887, vol. 178, p. 539, *et seq.*).

Reference to the descriptions which we have already given will show, that the external appearance, and distribution, of the nodules was very different on the roots of the peas, the vetches, and the lupins. In the case of the peas there were many of what may be called agglomerations of nodules, and comparatively few single ones distributed on the root-fibres. On the roots of the vetches, there

were comparatively few agglomerations or bunches, and more single nodules, pretty widely distributed along the root-fibres. The lupin roots, on the other hand, showed tubercular developments very different from those on either the pea or the vetch roots. Indeed, at the period of examination, that is when the plants were nearly ripe, two apparently distinct kinds were observed; one of which, the most prevalent, we have spoken of as "swellings," and the other as "nodules." The "swellings" were chiefly on the main roots or the thicker branches; where they grew they encased the root entirely, and they had a shining and presumably impervious skin. The "nodules," on the other hand, were chiefly single, small, and distributed on the root-fibres. Assuming that the so-called "swellings" were the bodies which, with their contents, had exercised the functions of the "nodules" found on the roots of the other plants, it is to be concluded that, after the very luxuriant growth, and the flowering and seeding, their function was so far at an end, and they had become suberised. The other bodies on the lupin roots, distinguished in the description as "nodules," indicated too meagre development to have had much share in the great amount of assimilation that had been accomplished. On the other hand, the "swellings," as has been said, were all on the main roots or thicker branches; whilst it is generally stated that the nodules are only formed on the young and still growing roots. If these "swellings," which were certainly very characteristic of the roots of the plants which attained the greatest growth, were really the effective nodules, it must be supposed that they had been formed where they were found, whilst the root was still young, and had grown with its growth. In favour of this supposition is the fact that the increased growth from the soil-extract seeding commenced quite early in the life of the plants.

In 1887, the nodule development on lucerne roots was observed at different periods of the season, and again quite recently, on plants taken from the field for that purpose. The nodules on the roots of lucerne are quite different in general external character from those on any of the other plants that have been examined at Rothamsted. Instead of being more or less rounded, they have more the appearance of shoots or buds, much longer than broad, sometimes single, but more often divided, or branched; there being generally two or three, and sometimes as many as twenty, or even many more, in a bunch, joined at the base. They have not been observed on the main root, but only on the root-fibres, and less near the surface than within the range of the clay subsoil. In some cases such a tuft or bunch will be at the end of a fine fibre by which it is connected with the main root. As the season advances these bodies become shrivelled, and are in fact empty shells. The question arises, whether in the case of the development in soil or subsoil containing organic nitrogen,

the lower organisms may not serve the higher, in part at least, by taking up, either directly or indirectly, combined nitrogen; as, for example, fungi take up organic nitrogen from the soil; or as, it may be assumed, does the fungus in the case of the fungus-mantle observed by Frank on the roots of Cupuliferæ, and some other plants?

Among the Leguminosæ growing in the mixed herbage of grass land, in 1868, nodules were observed on the root-fibres—of *Lathyrus pratensis*, especially near the surface of the soil; on the ultimate root-fibres of *Trifolium pratense*, and on the smaller rootlets of *Trifolium repens*.

In the case of red clover growing in rotation on arable land, an abundance of nodules has been found, both near the surface and at a considerable depth. They are generally more or less globular or oval. Some found on the main roots are more like "swellings" than attached tubercles, not, however, encasing the root, but only on one side. The greater number are, however, small, and distributed chiefly on the root-fibres. Observations are, however, needed, as to any difference in character, or relative prevalence, at different periods in the life and growth of the plant, and under different conditions of soil, both so far as mechanical state and porosity, and richness or otherwise in available supplies of combined nitrogen, are concerned. To these points we hope to pay some attention.

Referring to the main object of the investigation, it will be admitted that the results so far brought forward are abundantly confirmatory of those obtained by Hellriegel, and that the fact of the fixation of free nitrogen in the growth of Leguminosæ, under the influence of microbe seeding of the soil, and of the resulting nodule formation on the roots, may be considered as fully established.

It appears that, almost concurrently with the experiments made at Rothamsted, M. Bréal, of the Physiological Laboratory of the Muséum d'Histoire Naturelle, of Paris, has made various experiments on lines suggested by the results obtained by Hellriegel and Wilfarth. He examined the contents of nodules from lucerne roots, and observed rounded grains and bacteria-like filaments. He determined the nitrogen in the root-tubercles from various Papilionaceæ, and found it much higher in them than in the stalks, leaves, or roots. He germinated peas in a nutritive solution, and added some of the matter from a crushed lucerne root-tubercle. The pea roots became covered with tubercles, and eventually the nitrogen in the plant was about double that in the seed sown. In another experiment he germinated two lupin seeds, inoculated one of them from a living lucerne root-tubercle, and planted both in gravel with a nutritive solution free from nitrogen. Eventually the roots of the inoculated plant were covered with tubercles, whilst those of the other had

none. The inoculated plant also contained about two and a half times as much nitrogen as the seed, whilst without inoculation there was practically no gain. This experiment has been repeated by Hellriegel with very striking results, as one of us had the opportunity of seeing in August last. In another experiment, peas were germinated in a lucerne soil, transplanted into gravel, and nutritive solution free from nitrogen added, when the roots became covered with tubercles, and the nitrogen assimilated was nearly twenty-five fold that of the seed. On inoculating the germinated roots of haricots, and planting them in sand, they grew vigorously, formed pods, developed many tubercles on their roots, and assimilated nearly fifteen times as much nitrogen as the seed supplied. Lastly, he planted a fragment of lucerne root with nodules on it, in a sandy soil, reserving a similar fragment for analysis. Several cuttings of lucerne were obtained; and when taken up the root had many nodules, and the nitrogen assimilated was more than eighty times as much as in the root planted.

As to the importance to agriculture, in a quantitative sense, of this newly established source of nitrogen to the Leguminosæ, the evidence at present at command is insufficient to enable us to form any very decided opinion. Both agricultural investigation and direct vegetation experiment have clearly shown that Leguminosæ do take up much soil-nitrogen, and, at any rate in great part, as nitrate. But in our recent paper in the 'Philosophical Transactions' before referred to, we showed that, in some special cases, there was no evidence to justify the conclusion that the whole of the nitrogen had been so derived; and it was admitted that some other explanation of the large amounts of nitrogen assimilated was needed. It is not improbable that, in those cases, the agency now under consideration contributed to the result.

Then, as to the growth of leguminous crops in the ordinary course of agriculture. Hellriegel agrees with us that they do utilise soil-nitrogen, and he thinks probably always first; but that that source is supplemented by nitrogen brought into combination under the influence of the symbiotic growth of special organisms and the higher plant; and he supposes that the proportion of the total nitrogen assimilated which will be due to this latter source will be greater in crops grown in soils that are poor than in those which are rich in nitrogen. He considers it probable, however, that even in the case of rich soils there will be always more or less gain due to such fixation. The proportion of the nitrogen assimilated which will be gain depends, therefore, on complicated conditions. As bearing upon this subject it may be stated that, in experiments with beans, Professor Vines found that the formation of tubercles on the roots was very much reduced, if not indeed only accidental, when the plant was liberally supplied

with nitrate. Again, certainly the evidence of the experiments which have been described, so far as it goes, seems to indicate a less development of nodules when the soil contained an abundance of combined nitrogen. If this indication should be confirmed, and the inference be generally applicable, it would be concluded that the agency of the symbiotic growth supposed, in fixing free nitrogen, will, other things being equal, be the less the more the soil itself is in a condition to supply an abundance of combined nitrogen; whilst its capability in this respect will depend not only on the richness in combined nitrogen of the soil within the range of the roots, but on its state of combination, and on the character of the soil as to porosity and aëration. On the other hand, the development of the supposed nitrogen-fixing organisms obviously depends on the infection of the soil with the organism essential to symbiotic life with the particular leguminous crop to be grown. It would also seem that it is, at any rate in some cases, dependent on the due porosity and aëration of the soil.

Should these assumptions be borne out by the results of future investigation, we may conclude that the proportions in which any particular leguminous crop will derive its nitrogen from soil-supplies of combined nitrogen on the one hand, and from fixation under the influence of the symbiotic growth on the other, will be very different, according to the characters of the soil, as to available supply of combined nitrogen, mechanical condition, and due infection. We should further conclude that, in such cases as those in which poor sandy soils will not grow fair crops of cereals, but will nevertheless yield enormous crops of some leguminous plant—lupins, for example—the leguminous crop will depend for a large proportion of its nitrogen on fixation, under the influence of the symbiotic growth. Again, in such cases as those of the growth of lucerne for many years in succession, as in some Continental countries, it may be supposed that such fixation would be the source of a considerable proportion of the very large amounts of nitrogen assimilated over a given area under such conditions.

In the case of beans, there is evidence that there is nodule-formation when the plant is grown under ordinary conditions, in the garden or in the field; it has also been seen that nodules were formed on the roots of the peas and the vetches experimentally grown in garden-soil; and the inference so far is, that wherever there is such formation, there is more or less fixation.

Then, as to the important case of the growth of red clover in our rotations. There can be no doubt that red clover does avail itself of soil supplies of combined nitrogen. On the other hand, the so-called leguminous nodules have frequently been observed on the roots of red clover growing in the field. Further, although Hellriegel in his earlier experiments did not get definite results with clover, he has

subsequently obtained increased growth by seeding with extract from both a loamy humus-soil, and a root-crop-soil; but the result was less marked than with some other Leguminosæ. It has been seen that, in the first year of the experimental growth of clover at Rothamsted, no beneficial effect resulted from seeding with rich garden-soil-extract. It is believed, however, that the growth in the sterilised sand without soil-extract seeding will prove to be greater than can be accounted for by the supply of nitrogen in the seed sown. If this should turn out to be the case, the supposition will be that the necessary infection has come from the atmosphere. In reference to this point it may be mentioned that the glass-house in which the experiments are conducted stands in the midst of allotment gardens, in which a great variety of vegetables is growing, whilst Hellriegel's most definite result with clover was obtained by seeding with an extract from a root-crop soil.

Existing evidence is, therefore, in favour of the supposition that red clover does derive some of its nitrogen from fixation under the influence of proper soil-infection, and the resulting symbiosis of the lower and the higher growth. There is, however, at present very little definite evidence to guide us in judging under what conditions, on the one hand soil supplies of combined nitrogen, and, on the other such fixation, will contribute more or less of the total nitrogen of the crop. As one important element in forming a judgment on the subject, it is, as already said, our intention to study the conditions under which the development of nodules on the roots of growing clover is more or less favoured.

Upon the whole, then, the evidence at command points to the conclusion that, in the case of most if not all of our leguminous crops, a greater or less proportion of their nitrogen will be due to the fixation supposed.

Admitting the fact of such fixation to be fully established, the question still remains, how is it to be explained? Unfortunately, here again, as in the matter of the importance to agriculture in a quantitative sense, of this source of nitrogen to our crops, there is much yet to learn before a satisfactory answer can be given. Hellriegel frankly admits that a satisfactory explanation is still wanting; and we agree with him that we must know more of the nature and mode of life of the organisms which, in symbiosis with the leguminous plant, bring about the fixation of free nitrogen, before the nature of the action can be understood. As to the mode of life of these bodies, we owe much to the investigations of Marshall Ward, Prażmowski, Beyerinck, and others; but probably none will more readily admit than themselves, that the facts which they have established so far, are insufficient to afford an adequate explanation of the phenomena involved.

It is, it seems to us, a point of importance that it should be established, as it appears clearly to be, that in the development of the parasite the cortex of the host is penetrated, and so an intimate connexion between the two, indeed a symbiosis, is set up. Then there is abundant evidence that the nodules are very rich in nitrogen. So far as the facts at command go, it would seem that their dry substance may contain a higher percentage of nitrogen than that of any other part of the still growing plant; and, in some cases at any rate, even higher than in that of the highly nitrogenous leguminous seed itself.

Whence comes this nitrogen? The opinions of those who have specially studied the histology and biology of the subject, do not seem to be very clear or definite in reference to this point. According to Prażmowski, as quoted by Marshall Ward, the bacteroids "can only multiply in the still *living* protoplasm." Again, under the influence of the fungus—"the young tubercle is developed in the deeper parts of the cortex, and in its tissues the bacterium-like contents of the fungus become distributed, and grow, divide, and branch at the expense of the protoplasmic contents. He regarded the phenomenon as one of symbiosis, and as benefiting the host as well as the parasite." And again—"The tubercle-bacteria penetrate through young (not suberised) cell membranes into the root-hairs and epidermis cells of the root, and there multiply at the expense of the protoplasmic cell-contents."

Further, "The contents of the bacteroid cells are resorbed as the bacteroids dissolve, certain substances being left behind. In other words, the plant utilises the substance of the bacteria. When emptying begins, and with what energy it proceeds, depend especially on the quantity of nitrogenous compounds at the disposal of the roots. In a soil rich in nitrogen the tubercles go on developing unhindered, become large and typical, and rosy inside, and are not exhausted till late; in poorer soils they attain no great size, are soon emptied, and are green-grey inside."

Summarising the results and conclusions of Prażmowski, Marshall Ward says—

"No decision is arrived at as to whether the nitrogen is got from nitrogen compounds or from the free nitrogen of the air, nor as to what advantage accrues to the bacteria and the host-plant respectively." And again:—

"From the preceding, we see that the tubercles depend on a symbiosis which is advantageous to both the plant and the bacteria. The latter feed on the sap and cell-contents, and multiply through innumerable generations, and, both during the life of the host and afterwards, become redistributed in the soil. The plant derives advantage in that it obtains nitrogen by means of the bacteria.

Though the symbiosis is useful to both, the plant gains most, for it is the more powerful, and sooner or later overcomes the bacteria, to the multiplication of which it sets limits and finally absorbs the substance of the latter. Being the stronger, the plant directs the symbiosis."

If we understand the foregoing statements rightly, it is assumed that the bacteria acquire their nutriment, including their nitrogen, from the protoplasmic cell-contents of the higher plant; and that, on the other hand, the contents of the bacteroid cells are resorbed. "In other words, the plant utilises the substance of the bacteria." But it is obvious that, so far as the nitrogen of the bacteria is derived from the plant itself, the latter is not a gainer in a quantitative sense.

It is further assumed, that the activity of the process depends—"on the quantity of nitrogenous compounds at the disposal of the roots. In a soil rich in nitrogen the tubercles go on developing unhindered, become large and typical, ... in poorer soils they attain no great size, &c." Here, then, combined nitrogen in the soil is supposed to be the source of the nitrogen of the bacteria, and that they develop the more, the greater the supply of it. Undoubtedly, however, the nodules may develop very plentifully in a nitrogen-free soil, and there may be great gain of nitrogen, if only the soil be suitably infected. Indeed, the tendency of the evidence so far at command seems to show, that both the development of the nodules, and the gain of nitrogen, may be the greater in the poorer, but properly infected soil. Further, so far as the combined nitrogen of the soil is the source of the nitrogen there is no gain of it.

Marshall Ward says, however, that no decision is arrived at as to whether the nitrogen is got from nitrogen compounds or from the free nitrogen of the air, nor as to what advantage accrues to the bacteria and the host-plant respectively. But he adds that the symbiosis is advantageous to both the plant and the bacteria; the latter feeding on the sap and the cell-contents, whilst the plant obtains nitrogen by means of the bacteria.

It is obvious, however, that if the nitrogen of the bacteria is derived from the plant itself, it will be quantitatively no gainer by resorbing it. Nor would there be any such actual gain of nitrogen as there undoubtedly is, if the source of the nitrogen, either of the parasite or of the host, were essentially the supplies of combined nitrogen within the soil.

The most probable alternatives seem to be—1. That, somehow or other, the plant itself is enabled, under the conditions of the symbiotic life, to fix the free nitrogen of the atmosphere by its leaves; a supposition in favour of which there seems no evidence whatever. 2. That the parasite utilises and fixes the free nitrogen, and that the nitrogenous compounds formed are taken up by the host. On such a supposi-

tion, the actually ascertained large gain of nitrogen by the leguminous plant growing in a nitrogen-free, but properly infected, soil becomes intelligible. It is admitted, however, that further investigation of the mode of life of the parasite, especially having regard to its surrounding media, is needed.

It seems to us that there is nothing in the evidence pointing to the conclusion that the fixation is effected by the lower organisms within the soil independently of the symbiotic life. We do not here enter into the question, so much discussed of late, as to whether or not there is fixation within the soil under the influence of other low organisms, independently of the associated growth of a higher plant.

In our recent paper in the 'Philosophical Transactions,' before referred to, we said that whilst experience, whether practical or experimental, did not point to an unsolved problem in the matter of the sources of the nitrogen of the agricultural plants of other families, it was far otherwise so far as those of the Papilionaceæ were concerned. Further, that since the question of the sources of the nitrogen of the Leguminosæ had been the subject of experiment and of controversy for about half a century, and it was admitted that all the evidence that had been acquired on lines of inquiry previously followed had failed to solve the problem conclusively, it should not excite surprise that new light should come from a new line of inquiry; and, that hence should be recognised the importance of the cumulative evidence of the last few years, of which that furnished by the experiments of Hellriegel and Wilfarth was certainly the most definite and the most striking, pointing to the conclusion that although chlorophyllous plants might not directly utilise the free nitrogen of the air, some of them, at any rate, may acquire nitrogen brought into combination under the influence of lower organisms, the development of which was, apparently, in some cases a coincident of the growth of the higher plant whose nutrition they were to serve. It was added, that as such a conclusion was of fundamental and far-reaching importance, it was desirable it should be confirmed by independent investigation.

The results even so far obtained, and recorded in this paper, can leave no doubt that this important conclusion is confirmed, so far as a number of agricultural plants of the leguminous family are concerned. The question suggests itself, whether such, or allied agency, comes into play in the nitrogen assimilation of leguminous plants generally, or of that of other than the agricultural representatives of the non-leguminous families to which we owe such plants, or of those of the numerous and varied other families of the vegetable kingdom.

It is true that the families which contribute staple agricultural plants are but few, and that the agricultural representatives of those

families are also comparatively few. The families so contributing are, however, among the most important and widely distributed in the vegetable kingdom; as also are some of the plants they contribute. As prominent examples may be mentioned, the *Gramineæ*, affording the cereal grains, a large proportion of the mixed herbage of grass-land, and other products; also the *Leguminosæ*, yielding pulse crops, many useful herbage plants, and numerous other products. As we have said, there does not seem to be an unsolved problem as to the sources of the nitrogen of other of our agricultural plants than those of the leguminous family. Obviously, however, it would be unsafe to generalise in regard to individual families as a whole, from results relating to a limited number of examples supplied by their agricultural representatives alone. Still, there is nothing in the evidence at present at command, to point to the supposition that there is any fundamental difference in the source of the nitrogen of different members of the same family, such as is clearly indicated between the representatives of the leguminous, and of the other families, supplying staple agricultural products. On the other hand, existing evidence does not afford any means of judging whether or not similar, or allied agencies to those now under consideration, or even quite different ones, may come into play in the nitrogen assimilation of the members of other families which contribute such a vast variety of vegetation to the earth's surface.

We have pleasure in stating that the conduct of the investigation has largely devolved upon Dr. N. H. J. Miller. He has been almost wholly responsible for the analytical work, as well as for the photographing, by which a permanent record, not only of the aboveground growth, but of the root-development of the experimental plants has been secured. It should be added, that Mr. J. J. Willis has materially assisted in the observation and noting on growth; also in the separation of the roots, mounting them for observation and for photographing, and in noting upon them.

II. "On Electric Discharge between Electrodes at different Temperatures in Air and in High Vacua." By J. A. FLEMING, M.A., D.Sc., Professor of Electrical Engineering in University College, London. Communicated by Professor G. C. FOSTER, F.R.S. Received December 16, 1889.

(Preliminary Notice.)

It has been known for some time that if a platinum plate or wire is sealed through the glass bulb of an ordinary carbon filament incandescent lamp, this metallic plate being quite out of contact with